Seasonal Gonadal Changes of Adult Oviparous Oysters in Tomales Bay, California

BY

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(Plates 1 to 3; 1 Text figure)

INTRODUCTION

Two species of oviparous oysters, *Crassostrea virginica* (Gmelin, 1791), the Eastern oyster, and *Crassostrea gigas* (Thunberg, 1793), the Japanese oyster, were introduced into Tomales Bay, California for commercial purposes. Because these oysters do not reproduce in its waters, shipments of adult oysters or oyster spat must be periodically transplanted into this bay. It is the purpose of this study to compare the seasonal gonadal changes which occur in the two species of adult oysters in Tomales Bay with one another, and to compare them with similar changes occurring in the same species of oysters in other regions. The differences in gonadal changes will be related to variations in environmental conditions and to specific or racial characteristics.

Tomales Bay is located on the Northern Californian Coast, approximately 40 miles north of San Francisco. The oysters used in this study were kept at Tomales Bay Oyster Company, which is situated in the cove between Millerton and North Double Point, near the headwaters of the bay. This area has long been the site of oyster culture. The native or Olympia oyster (*Ostrea lurida* Carpenter, 1864) was the first oyster species grown in Tomales Bay to be shipped to the markets of San Francisco. However, upon completion of the transcontinental Central Pacific Railroad in May 1869, it was possible for the first time to ship live adult *Crassostrea virginica* and its seed to the Pacific coast. In 1875, 17 carloads of *C. virginica* were laid out near Millerton Station in Tomales Bay (Townsend, 1893). Cultivation of *C. virginica* in Tomales Bay has continued intermittently since that time. The tidelands of Tomales Bay Oyster Company were also the first areas in California to be used for the culture of Japanese oysters (*C. gigas*), which were introduced into Puget Sound in 1902, and later, in 1928, into Tomales Bay (Barrett, 1963). Both *C. virginica* and *C. gigas* have failed to reproduce in Tomales Bay and continued importation of adult oysters or seed has been necessary.

Because of their commercial value, there have been numerous attempts at the artificial introduction of oysters into areas lacking natural populations, or in which the native oyster is of little economic importance. The oysters of the genus *Crassostrea* Sacco, 1897, are more often used as introduced species than are those of the genus *Ostrea* Linnaeus, 1758, since they have a greater ability to survive in extreme and varying environments. *Crassostrea virginica* has been introduced into the oyster beds of Britain, the Pacific Coast of North America, and the island of Oahu, Hawaii. *Crassostrea gigas* has likewise been introduced into the oyster beds of the Pacific Coast of North America; Oahu, Hawaii; also into Melbourne Harbour, Australia; Mobile Bay, Alabama; and Barnstable Bay, Massachusetts. The Portuguese oyster, *C. angulata* (Lamarck, 1819) has taken over all of the French oyster beds and now also lives, but does not reproduce, in British beds. One noteworthy and successful introduction of oysters of the genus *Ostrea* took place in the waters of Boothbay Harbor, Maine, where Loosanoff (1955) had transplanted European oysters (*Ostrea edulis* Linnaeus, 1758) in 1949.

In many of the attempts to introduce a species of oyster to a new location, the animals may have survived and grown well, but failed to reproduce. To determine the reason for the failure of the species to reproduce, one must be familiar with the seasonal gonadal changes which occur in the oysters in their native environment. There have been studies on gonadal changes in each of the commercially valuable species of oysters. *Crassostrea*

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virginica is the most extensively studied species of the oviparous oysters (Hopkins, 1931; Loosanoff, 1932, 1942, 1965; Loosanoff & Engle, 1942; Butler, 1949; Kennedy & Battle, 1964; Sevilla & Mondragon, 1965). *Crassostrea gigas* has had little work done on its gametic activity (Imai *et al.*, 1950; Imai & Saki, 1961) and the same is true with the other oviparous oysters: *C. commercialis* (Iredale & Roughley, 1933) (Roughley, 1933; Cleland, 1947); *C. angulata* (Bargeton, 1942, 1943); and *C. madrasensis* (Preston) (Anonymous, 1950). Studies have also been made of gonadal changes in the larviparous oysters: *Ostrea edulis* (Orton, 1927, 1931, 1933; Cole, 1942) and *O. lurida* (Coe, 1931, 1932).

Even less work has been done on the seasonal gonadal changes in oysters which have been introduced into new areas. Loosanoff (1962a) gave a detailed description of the changes which occurred in the gonads of *O. edulis* introduced into Boothbay Harbor, and briefly mentioned the gonadal changes which occurred in *C. gigas* transplanted into Milford Harbor (Loosanoff & Davis, 1963). Galtsoff (1929) and Katkansky & Sparks (1966) discussed the gonadal changes and sex ratios which occurred in *C. gigas* cultured in the waters of the State of Washington, and Elsey (1932, 1933, 1934) described the changes in *C. gigas* in the waters of British Columbia. *Ostrea lurida* is the only other species in which the seasonal gonadal changes have been thoroughly studied in both the native environment (Coe, 1931, 1932) and in the area to which it was introduced (Hori, 1933).

The introduction of oysters into a new environment is valuable for both scientific and commercial reasons. Although it has long been known that *Crassostrea virginica* and *C. gigas* do not reproduce in Tomales Bay, no one has ever done a detailed study of the reproductive cycle or the seasonal histological changes in the gonads. This study, therefore, extends the knowledge concerning an oyster's adaptation to its new environment and may help to define the reasons for the failure of these two species of oysters to reproduce in Tomales Bay.

MATERIALS AND METHODS

The oysters used in this study were obtained from Long Island Sound and from Canadian waters through the courtesy of the Department of Fish and Game of the State of California, Dr. Victor L. Loosanoff and Dr. Edmund H. Smith of the Pacific Marine Station. Four hundred and fifty 2-year old *Crassostrea gigas* of Canadian origin were taken from the mud-flats at Tomales Bay Oyster Company where they had been raised. They were then placed in wire-mesh trays and suspended from racks at the Oyster Company on October 20, 1966. A similar number of 2-year old *C. virginica* was received on October 26, 1966, and was placed in identical adjacent trays. Fifteen oysters of each species were collected at bi-weekly intervals for 15 months. An additional sample of 13 *C. virginica* was obtained on October 5, 1966, from a stock which had been previously kept at Tomales Bay Oyster Company.

The height (distance between the umbo and the ventral valve margin) and length (distance between anterior and posterior valve margins) of each oyster were measured with calipers before the oyster was opened. A tissue sample was taken from the gonadal area near the labial palps in conformity with the practice of the biological laboratories of the United States Bureau of Commercial Fisheries. This gonadal tissue was fixed in Bouin's solution, infiltrated with 52.5° C paraffin, sectioned at 10 μ, and stained with Heidenhain's iron hematoxylin and aqueous eosin Y, using standard procedures. The tissues were then microscopically analysed for sex and state of gametogenesis.

Hydrographic data were collected on each sampling date; they included measurements of salinity, hydrogen-ion concentration, and turbidity of water samples taken one foot above the bottom by means of a Frautschy-bottle sampling device. Sub-surface water temperatures were taken by suspending a thermometer over the bottom. Surface-water hydrographic data were obtained from records kept at the Pacific Marine Station.

Large bags of sun-bleached oyster shells were suspended near the trays of oysters for 2-week periods from May 4, 1967 until October 19, 1967. Fifty shells were chosen at random from each bag and examined for oyster spat. During the same period plankton tows were taken over the beds of Tomales Bay Oyster Company and examined for bivalve larvae. All oyster larvae were identified by length-width measurements after the method of Loosanoff, Davis & Chanley, 1966.

OBSERVATIONS

A detailed description of the sequence of events in the development of functional gametes has been given by Loosanoff (1942) for *Crassostrea virginica.* Since the seasonal gonadal changes observed in both *C. virginica* and *C. gigas* in Tomales Bay differed little from those described, except with regard to timing (Loosanoff & Engle, 1940; Loosanoff, 1965), only a résumé of the gonadal changes will be given here. It is most convenient to describe the seasonal gonadal changes which occurred

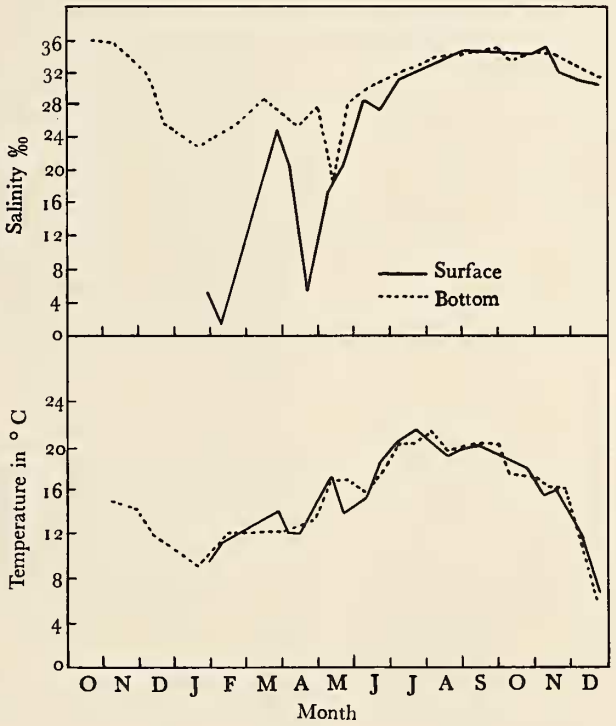


Figure 1
Water temperature and salinity at Tomales Bay Oyster Company
from October 1966 to December 1967

in the two species separately and in the order in which they were observed.

Specimens of *Crassostrea virginica* were obtained from Tomales Bay Oyster Company on October 5, 1966, where they had been kept for at least a year. The small gonadal follicles of these oysters contained only indifferent sex cells and were scattered in the large masses of connective tissue. This "indifferent stage" (Loosanoff, 1942, p. 203) was characteristic in the months of September, October, and November of 1967.

The remaining samples of gonadal tissue were collected from *Crassostrea virginica* transplanted into Tomales Bay Oyster Company beds on October 26, 1966. Slight gametic activity ended the indifferent stage of some of the oysters during the months of November and December. Primary and secondary gametogonia started to develop along the follicular walls, making sex determination possible. The follicles began to expand gradually.

A marked increase in the size of the follicles and in the acceleration of maturation of the gametes was noticed in late December and January. Ovocytes began to fill the follicles and spermatids were already developing. The follicles showed rapid growth and ramification.

Continued maturation of the gametes took place in February and March, concurrently with the disappearance of the voluminous vesicular connective tissue. In some instances, mature ova and spermatozoa were present.

By the sixth of April, most of the gonads were packed with ripe gametes, some of which nearly filled the ciliated genital ducts (Plate 1, Figures 1 and 2). However, a few cells at the earlier stages of gametogenesis were present on the follicular walls. This state of maximum ripeness was maintained throughout the spring. Although a few oysters were partially spawned beginning at the middle of April, mass spawning did not occur until the middle of June and continued until the middle of July. On July 13, 87% of the sample was completely spawned. This coincided with the time of greatest rate of increase in water temperature (Text figure 1).

The gonads of spawned oysters were characterized by the absence of mature gametes and the shrunken appearance of the follicles (Plate 1, Figures 3 and 4). Great numbers of phagocytic cells were present, both inside the lumina of the follicles and around the outside walls. All unshed gametes are devoured by these cells. The lumina of the follicles were being closed by elongation and the shrinking of follicular tissue. Simultaneously, the cells of the vesicular connective tissue proliferated, filling all interfollicular spaces. Resorption of gametes continued from the post-spawning stages in July until the first week of November, and a few follicles were observed to contain mature gametes being phagocytized as late as December. However, most gonads were in the indifferent stage by September.

All samples of gonadal tissue from *Crassostrea gigas* were collected from oysters which had been raised in Tomales Bay from the imported seed. Although the seasonal gonadal changes which were observed in *C. gigas* resembled those of *C. virginica,* there were differences between species in timing and in the homogeneity of the sample. The indifferent stage of gametogenesis was characteristic of the months of November and December.

The little gametic development and proliferation of the follicles which occurred in December and January is the only activity which might be characterised as the "sex-differentiation stage" (Loosanoff, 1942, p. 203).

Very rapid maturation of the gametes took place in January and February, with a great expansion of the follicles. March was the month of greatest maturation and proliferation. By the eleventh of March, ripe ova and spermatozoa were present in a few of the follicles.

From April 6 until July 27, 95% of all the gonads sampled were filled with ripe gametes (Plate 2, Figures 5 and 6). They retained gametes throughout the spring, however, because no spawning took place until after the twenty-seventh of July. Between the dates of July 27 and August 10, mass spawning of *Crassostrea gigas* occurred. This coincided with the warmest water temperatures to that date (Text figure 1). Again, the follicles of the spawned gonads were shrunken and devoid of ripe gametes (Plate 2, Figures 7 and 8).

Resorption and cytolysis of the unshed gametes started immediately after spawning was completed and con-

Explanation of Plate 1

Figure 1: Section of gonad of ripe female *Crassostrea virginica* collected April 6, 1967 (× 125)

Figure 2: Section of gonad of ripe male *Crassostrea virginica* collected April 6, 1967 (× 125)

Figure 3: Section of gonad of spawned female *Crassostrea virginica* collected July 13, 1967 (× 125)

Figure 4: Section of gonad of spawned male *Crassostrea virginica* collected July 13, 1967 (× 125)

Explanation of Plate 2

Figure 5: Section of gonad of ripe female *Crassostrea gigas* collected April 6, 1967 (× 125)

Figure 6: Section of gonad of ripe male *Crassostrea gigas* collected April 6, 1967 (× 125)

Figure 7: Section of gonad of spawned female *Crassostrea gigas* with a few ova remaining in gonad tubules. collected August 10, 1967 (× 125)

Figure 8: Section of gonad of spawned male *Crassostrea gigas* showing spermatozoa remaining in gonad tubules. collected August 10, 1967 (× 125)

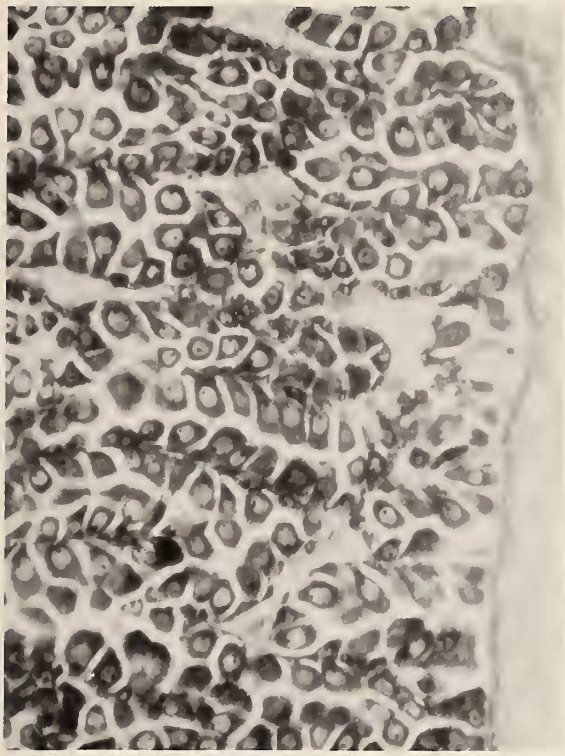
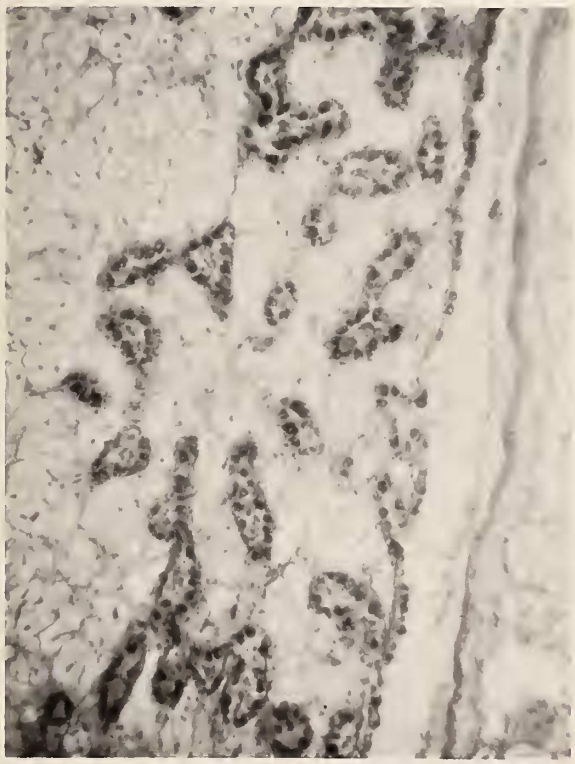


Figure 1

Figure 2



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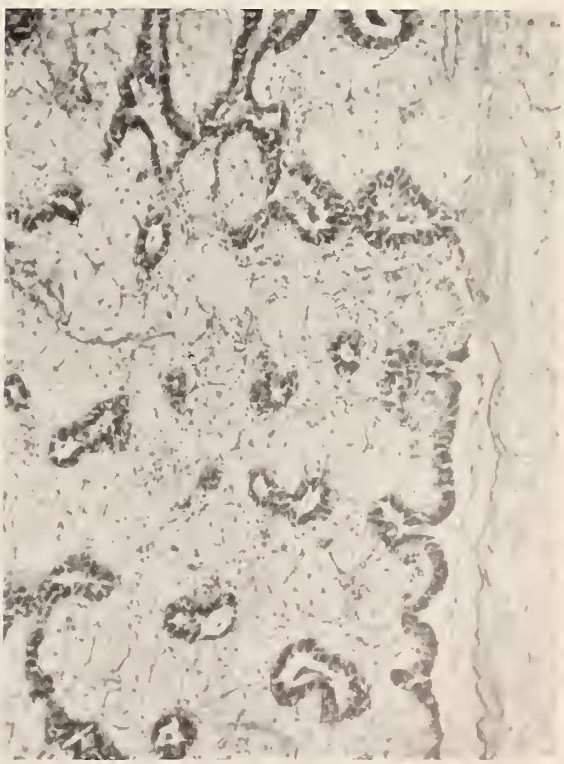

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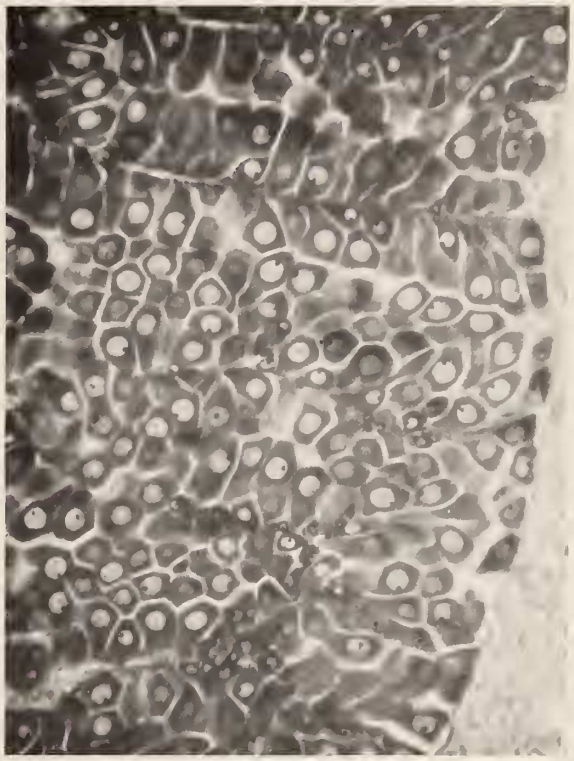

Figure 5

Figure 6

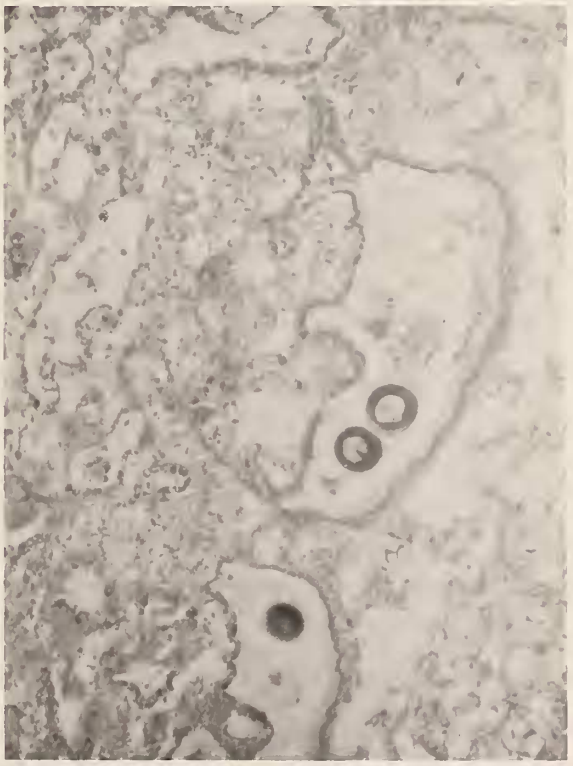

Figure 7

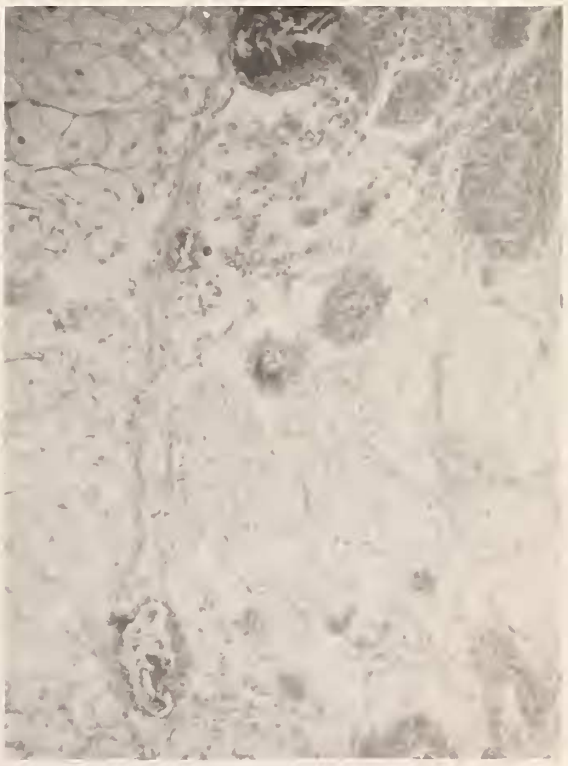

Figure 8

tinued until December. Many spermatozoa and ova were found being phagocytized, some until late December.

In addition to observing the gonadal changes, the sex ratio was also noted. Frequently, however, the sex could not be determined since the gametes were not yet differentiated. In a sample of 387 *Crassostrea virginica,* 139 were male, 114 were female, and 132 were undifferentiated. Two morphological hermaphrodites were found. Both contained follicles packed with spermatozoa and a few cytolized ova (Plate 3, Figure 9). In a sample of 382 *C. gigas* 100 were male, 194 were female, and 87 were undifferentiated. Only one hermaphrodite was found for this species. Unlike those observed in the sample of *C. virginica,* the follicles of the hermaphroditic *C. gigas* contained both male and female gametes in various stages of development (Plate 3, Figure 10).

The hydrographic conditions observed in the bay are presented in graphic form (Text figure 1). The range of hydrogen-ion concentration was not great enough (7.70 to 8.16) to affect gonadal changes, neither did it exhibit seasonal variances. Hence, these data are not presented in this article. The turbidity determinations are not presented either, although the turbidity of the water showed distinct seasonal variations. Both temperature and salinity may affect gametogenesis (Kinne, 1963, 1964; Loosanoff, 1945, 1948). Text figure 1 presents temperature and salinity data in graphic form so that seasonal trends may be more apparent.

The difference between surface and bottom salinity samples is significant and noteworthy since the oysters were exposed to surface water at low tides. The extreme range in salinities is due to the effect of fresh-water runoff during the winter and spring, and to evaporation during the summer. Salinities in excess of 32‰ prevailed from July to December. Throughout the winter and spring, however, the salinity remained around 25‰.

The water temperature at the oyster beds exhibited similar seasonal fluctuations. A trend of rising temperatures starts in January after a brief cold period and continues until June. The water temperature remains close to 20° C during June, July, August and September. The water then begins to cool to its lowest temperature at the end of the year. Rarely did the temperature remain below 10° C for more than a few days, neither did it range much above 20° C. All hydrographic observations, including temperature determinations, were made at approximately mid-tide. More extreme temperatures may exist during slack tides, but thermograph records for that area showed that the water temperatures remain markedly constant throughout the tidal cycle.

Finally, few *Crassostrea* larvae were collected by the plankton tows and few spat were found on shells. Some straight-hinge stage larvae were found in the plankton samples of August 24 which had general appearances and dimensions similar to those reported for *C. virginica* and *C. gigas* (Loosanoff, Davis & Chanley, 1966). Although bivalve larvae were collected in every sample, no others approximated the proper size or shape.

A total of 6 oyster spat was found set on shells; one on July 13, and 5 on August 10. Three of those found on August 10 had settled on the inside of the right valve of an oyster which was gaping. The inside of the shell was free of mud, although the outside was covered. Throughout the summer, all shells in the wire-mesh bags were found to be covered with mud after 2 weeks in the water. It is surprising that any larvae were able to set on such shells.

DISCUSSION

Gametogenesis and spawning of oysters are directly correlated to water temperatures. Loosanoff & Davis in 1952 have shown the temperature and time requirements to condition *Crassostrea virginica* to spawn. Their experiments showed that 10° C was not high enough to induce gametic activities. However, they report ripening and spawning for oysters when the temperature had reached only 15° C. Later experiments (Loosanoff, 1958, 1969) proved that maturation of gametes was possible after 68 days of conditioning at 12° C. The water temperatures at Tomales Bay Oyster Company remained above 12° C throughout the year, except for short periods of time in December and January. During the summer, the water temperature was not as high as those reported for either Long Island Sound or the Miyagi Prefecture, Japan, from where the oysters originally came. The differences in seasonal temperature fluctuations between Tomales Bay and the native environments of the oysters were responsible for the variances in gonadal changes (Table 1).

Low salinities which may have an influence on gonad development (Loosanoff, 1948; 1952), were not encountered for extended periods of time, thus they did not seem to affect gonadal changes in oysters in Tomales Bay.

A comparison of seasonal gonadal changes between *Crassostrea virginica* and *C. gigas* must take into account differences between the species and racial variations within each species. As mentioned previously, the seasonal gonadal changes observed in both *C. virginica* and *C. gigas* differed little from each other, except for timing. The differences in timing are clearly portrayed in Table 1. The only time of agreement in stages of gametogenesis was from the first week in April, when approximately 75% of the samples from both species contained ripe ova and

Table 1

Periods of Basic Gonadal Stages in *Crassostrea virginica* and *Crassostrea gigas* in Different Geographic Areas.

Stage	*Crassostrea virginica*		*Crassostrea gigas*		
	Tomales Bay California	Long Island Sound	Tomales Bay California	State of Washington	Miyagi Prefecture, Japan
Spring Development	January and February	April and May	February and March	May and June	—
First Ripening	April	June	April	May	May
Spawning	April through July	June through August	July and August	July and August	August and September
Resorption	From Post-Spawning to October	From Post-Spawning to October	From Post-Spawning to December	From Post-Spawning to December	—
Undifferentiated	September through November	August and September	November and December	October through February	—
Fall Gametogenesis	November and December	October and November	December and January	December and January	—
Inactive	None	December to April	None	February through April	—

sperm, until the end of June when most of *C. virginica* had spawned. During this period, no *C. gigas* spawned, but a few of *C. virginica* had partially spawned and some were undergoing resorption. Mass spawning of *C. virginica* was completed at least two weeks before *C. gigas* spawned, and it extended over a greater period of time than did the 2-week mass spawning of *C. gigas*. Since the shed gametes of both species can induce spawning in the males of either species (GALTSOFF, 1964), it is surprising that *C. gigas* did not spawn simultaneously with *C. virginica*. A combination of thermal and chemical requirements is often needed to induce spawning. These requirements were obviously not met for *C. gigas* until a later date.

Not all of the oysters of either species had spawned completely. More of *Crassostrea gigas* contained ripe ova and spermatozoa, and retained them until later dates, than did *C. virginica*. Resorption of unshed gametes continued into December for *C. gigas*. During and following resorption, the gonadal follicles were in the undifferentiated stage. This stage was halted with the advent of gametogenesis. In *C. virginica*, gametogenesis started in November, one month earlier than in *C. gigas*. Maturation proceeded gradually until after the first of the year for both species. In late December, January, and February, the gametes of *C. virginica* showed a great acceleration in maturation. This acceleration, termed "spring development" by LOOSANOFF (1942, p. 198), again occurred one month earlier in *C. virginica* than in *C. gigas*. However, *C. gigas* showed a greater burst of gametic activity in March. Thus, both species reached sexual maturity at the same time. In general, *C. gigas* has a higher temperature requirement for the initiation of both gametogenesis and spawning. This is evident by the slower development and later spawning dates. Moreover, unshed gametes are retained by *C. gigas* for longer periods of time before being resorbed. Similar differences were observed between *C. virginica* and *C. gigas* transplanted into Milford Harbor (LOOSANOFF & DAVIS, 1963).

A series of observations on seasonal gonadal changes of two other bivalve species was conducted concurrently with this study (LEONARD, 1969). *Ostrea edulis*, transplanted into Tomales Bay Oyster Company beds, and *Pododesmus cepio* (GRAY, 1850), the native rock jingle, both showed gonadal changes at dates which correspond well with those observed for *Crassostrea virginica* and *C. gigas* and with the seasonal fluctuations in temperature.

A comparison of the timing of the gonadal changes of *Crassostrea virginica* in Tomales Bay with those reported for it in Long Island Sound (LOOSANOFF, 1942; 1965)